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Anti-diabetic and Anti- Inflammatory activities of *Melia azedarach* extract

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Abstract

Diabetes mellitus is a metabolic disorder marked by persistently high blood glucose levels, which may result in significant morbidity and mortality. Individuals who are diagnosed with diabetes must take insulin for the rest of their lives and may experience several disease-related complications. Inflammation is considered a root of many disorders; it amplifies the production of electrons which results in higher production of oxygen reactive species. This leads to chain reactions that cause a great deal of ailments, including diabetes, cancer, and cardiovascular issues. Natural substances are way better than pharmaceutical drugs as they have many side effects along with curative properties. Plant extract and their secondary metabolites have traditionally been used in ethno-medicine to treat a variety of diseases. The primary objective of this study was to assess the anti-diabetic and anti-inflammatory effects of *Melia azedarach* extract. The anti-diabetic activity was evaluated using the glucose uptake by yeast cells assay, which demonstrated 70.03% activity at a concentration of 100 µg/ml. Additionally, its anti-inflammatory potential was examined through the red blood cell membrane stabilizing assay, yielding a result of 71.95% at 100 µg/ml. All these anti-diabetic and anti-inflammatory assays have revealed noteworthy results that support the traditional use of *Melia azedarach* as medicine. However, further *in vivo* study is needed with the extract of *Melia azedarach* its therapeutic effect against diabetes mellitus and Inflammation.

Keywords: Anti-diabetic and Anti- Inflammatory, plant extract

1.0 INTRODUCTION

Worldwide, a wide range of illnesses are treated with medicinal plants. Medicinal plants have biological uses because they contain a wide variety of chemicals. A significant source of natural products that are created with care and at a reasonable price is medicinal herbs [1]. They are mostly found in the tropics of Arabia, Africa, Zanzibar, the Mascarene Islands, Madagascar, and the southwest of India [2]. For hundreds of years, people have used plants as beneficial ingredients in medicines, cosmetics, fragrances, beverages, and colors to prolong their lives and keep them healthy [1]. The idea that plants inherently can heal and promote health is the cornerstone of herbal therapy [2]. The current focus on plant-based research has generated substantial data highlighting the significant potential of medicinal plants used in various traditional systems. [3]There is presently considerable public interest in the use of herbal treatments [3]. Among the widely used plants in traditional medicine is *Melia azedarach*, commonly referred to as melia, chinaberry, or cinamomo. Native to West Asia, this plant is known for its multiple therapeutic properties [4]. A near relative of Neem, *Melia azedarach* is a small to medium-sized deciduous tree or shrub that grows 5 to 15 metres tall [4]. It is found across tropical and subtropical regions and has phytochemical components that make it a potential for pest management.[5] It has been demonstrated to have nematicidal, insecticidal,[6] and antibacterial effects. Additionally, it is well recognised for its spermicidal, antipyretic[7], anti-inflammatory, anti-fertility, and anti-ulcer properties [5]. *Melia azedarach*, also known as Indian Lilac or Pride of India, is often grown in the Himalayan regions of countries like Pakistan, China, Burma, Bangladesh, and India [6]. Numerous scientific studies have documented various biological activities of *Melia azedarach* (MA), including its anticancer, antimalarial,[4] analgesic, anti-inflammatory, anthelmintic, antilithic, and diuretic effects [7]. Additionally, extracts from different parts of *Melia azedarach* have been reported to possess antifungal, anthelmintic, nematicidal, diuretic, cytotoxic, antiproliferative,[8] insecticidal, and antioxidant properties [8].Additionally, the use of anti-inflammatory medications has a variety of negative side effects, making the management of chronic inflammatory disorders complicated [9].

2.0 EXPERIMENTAL

2.1 Plant selection and extraction

Melia azedarach plants were collected in April 2023 from the Kalabat region in Swabi District, Khyber Pakhtunkhwa province, Pakistan. The plant sample was identified by Dr. Muhammad Ilyas from the Department of Botany, University of Swabi.

2.2 Extraction

The plant parts were dried for ten days at room temperature. The plant matter had been dried and ground into a fine powder. After this the plant parts were crushed up and soaked in methanol for five days, all of the plant compounds were extracted. The extracts were then concentrated using a rotating evaporator and lowered pressure at a temperature below 50 °C.[9]

2.3 Biological Activities

2.3.1 Invitro Antidiabetic Assays

Glucose uptake by Yeast cells Assay

The invitro antidiabetic activity of the plant extract was checked by Glucose uptake assays. Yeast cells are used as a model for diabetes because of their affinity for absorbing glucose. The glucose returns to the bloodstream when the insulin is unable to connect with the cells. The same methodology was applied in this test. with a slightly modified the experiment was carried out which described bt [10].

Yeast cells show a preference for glucose. Baker's yeast was obtained from the market and repeatedly centrifuged using distilled water to cleanse it. Until a transparent type of supernatant fluids emerged, the centrifugation process was maintained. A 10% (v/v) colloidal suspension of the pellet was then prepared in distilled water. To this suspension, 1 mL of a 5 mM glucose solution was added along with various concentrations of the test compound. The mixture was incubated at 37°C for ten minutes.

The reaction was initiated by adding yeast suspension. After vortexing, the mixture was incubated for an additional hour at 37°C. Following centrifugation at 3800 rpm for five minutes, the residual glucose in the precipitate was measured. Glucose absorption was then calculated using a UV5100B spectrophotometer.

The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\% \text{ activity} = \frac{(\text{control absorbance} - \text{extract absorbance})}{\text{control absorbance}} \text{ X } 100$$

2.3.2 *In-vitro* anti-inflammatory activity

The anti-inflammatory properties of the extract were evaluated using the human RBC membrane stabilization assay. The anti-inflammatory properties of the extract were assessed by measuring HRBC activity. We used sodium chloride (NaCl), purified water, fresh human blood, PBS (pH 7.4), and diclofenac sodium for this activity. A range of inflammatory diseases brought on by the lysosomal enzymes released during inflammation. The non-steroidal medication acts by stabilising or inhibiting the lysosomal membrane. Given that the lysosome and HRBC composition membranes were comparable [11]. Therefore, it was anticipated that the study will look at the HRBC membrane's stability to forecast the anti-inflammatory activity in vitro. New blood was drawn from the human who was in good health.[10]

Blood was combined with the anticoagulant EDTA and centrifuged in a falcon tube at 3000 rpm for 15 minutes. The remaining material was split once the supernatant was poured off. We used an isosaline solution (w/v) to clean all that remained. To obtain a clean supernatant, three centrifugation and washing cycles were necessary. An isotonic saline solution was used to create a 10% suspension using the pellet containing HRBCs.

The reaction procedure used in this experiment is as follows

Reaction Mixture: The control group, standard, and test samples were prepared using the reaction process that is described below.

Control group (4.5 mL): One milliliter of PBS, half a milliliter of packed cells, one milliliter of isotonic solution, two milliliters of hypotonic solution, and ten percent HRBC suspension were all included in the reaction mixture.

Standard group (4.5 mL): The standard group was prepared by adding 1 mL of isotonic solution, 2 mL of hypotonic solution, 0.5 mL of packed cells, 10% HRBC suspension, and 1 mL of diclofenac sodium to the reaction mixture at different concentrations (1:1).

Test group (4.5 mL): The test samples' reaction mixture group comprised of one milliliter PBS, half a milliliter packed cells with 10% HRBC suspension, one milliliter isotonic solution, two milliliter hypotonic solution, and one milliliter test sample containing varying concentrations (1:1) of (10, 20, 40, 60, 80, and 100 µg).

Incubation and centrifugation. Each reaction was incubated at 37°C for 30 minutes, followed by centrifugation at 3000 rpm for 15 minutes.

Spectrophotometry: The ultra-violet 5100B spectrophotometer were used to analyse the centrifuged supernatants at 560 nm. The following formula was use for the calculation of the percentage of hemoglobin denaturation, and all tests were run using triplicate readings.

$$\% \, Inhibition = \frac{Control \; Abs - Sample \; Abs}{Control \; Absorbance} x \; 100$$

3.0 RESULTS AND DISCUSSION

3.1 Anti-Diabetic

To study the effect of *Melia azedarach* on the glucose uptake by yeast cells in 5 mM glucose solution, various concentrations of *Melia azedarach* were used that are 10, 20, 30,40, 50,60,70, 80,90 and 100 µg which showed % glucose uptake of 6%, 11 %, 17 %,22 %, 28 %,37.4%,44%,52.9%,61%and 70.03% respectively. As a standard group, acarbose was utilized at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100µg/ml. The corresponding glucose uptake was found to be 11.2%, 19. %, 25%, 33%, 39.2%, 45%, 50%, 60%, 67%, and 78% (Fig. 1).

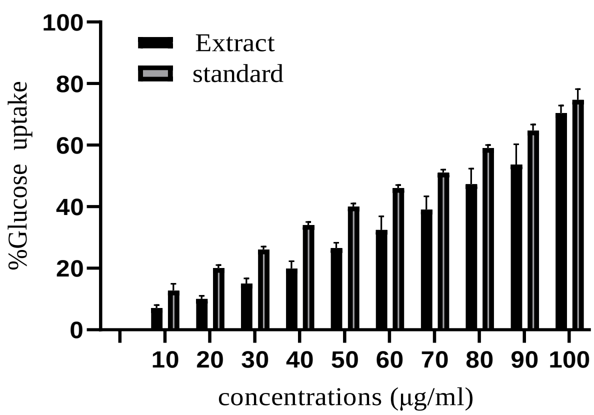

Figure 1. Antidiabetic activities of *Melia azedarach* extract

3.2 Anti-Inflammatory Effect of *Melia azedarach*

The HRBC-membrane stabilization assay was employed to evaluate the extracts in vitro anti-inflammatory activity.

Human RBC effect of *Melia azedarach*: The extract was used at varying concentrations of 10, 20, 40, 60, 80, and 100 µg, and the results indicated that the inhibition was 9.55 %, 19.77 %, 37.13%, 55.25%, 64.23%, and 71.95%, respectively. As a standard group, the drug diclofenac sodium was employed at 10, 20, 40, 60, 80, and 100µg/ml. The results showed that, accordingly, the inhibition was 32.66%, 54.23%, 67.38%, 74.57%, 80.95%, and 85.71%. Melia azedarach had a minimum inhibition rate of 9.55% at 10 µg. Conversely, the maximum inhibition rate of 71.95% was observed at 100µg as shown in Fig 2.

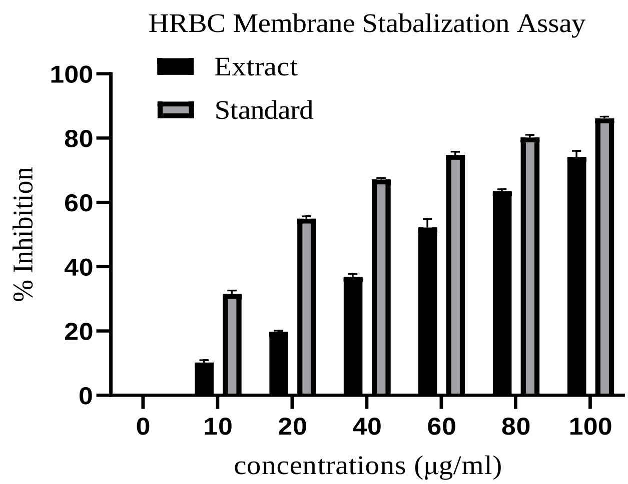

Figure 2. HRBC membrane stabilization assay of *Melia azedarach*

4.0 CONCLUSION

The findings of this study substantiate the traditional medicinal use of Melia azedarach extract for its anti-diabetic and anti-inflammatory properties. The extract exhibited significant glucose uptake activity, supporting its potential as an anti-diabetic agent, and demonstrated promising anti-inflammatory effects through membrane stabilization. These results highlight Melia azedarach as a natural source with potential therapeutic benefits against diabetes mellitus and inflammation. However, further vivo research is warranted to confirm these effects and explore their full therapeutic potential.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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